REMARKS

Claims 1-37 are pending in the application. Claims 8, 12, 14, 17, and 25 are canceled. Accordingly, claims 1-7, 9-11, 13, 15, 16, 18-24 and 26-37 remain under examination in the case. Applicants respectfully request reconsideration of the outstanding rejections for the reasons that follow.

Claim Amendments

Claim 1 is amended to recite a "conjugate consisting essentially of at least one antibody fragment covalently attached to no more than about 2 nonproteinaceous polymer molecules, wherein the apparent size of the conjugate is at least about 500 kD, wherein the antibody fragment comprises a heavy chain and a light chain corresponding to a portion of a parental antibody, wherein in the portion of the parental antibody the heavy and light chains are covalently linked by a disulfide bond between a cysteine residue in the light chain and a cysteine residue in the heavy chain, wherein in the antibody fragment the cysteine residue in the light or heavy chain is substituted with another amino acid and the cysteine residue in the opposite chain is covalently linked to a nonproteinaceous polymer molecule, and wherein at least one antibody fragment comprises an antigen binding site that binds to a polypeptide selected from the group consisting of: human vascular endothelial growth factor (VEGF), human p185 receptor-like tyrosine kinase (HER2), human CD20, human CD18, human CD11a, human IgE, human Apo-2 receptor, human tumor necrosis factor-α (TNF-α), human tissue factor (TF), human α4β7 integrin, human GPIIb-IIIa integrin, human epidermal growth factor receptor (EGFR), human CD3, and human interleukin-2 receptor α-chain (TAC)", as supported, at least, on page 53, line 9 to page 56, line 28 and page 66, line 17 to page 70, line 14 of the specification, and in original claims 1, 12, and 14.

Claims 9 and 15 are amended to be consistent with the claim cancellations and amended claim 1.

No new matter is believed to be added by the present claim amendments.

Drawing Informalities

The Office notes that the application was filed with informal drawings, and that color drawings and photographs will only be accepted upon Applicants' compliance with the applicable rules. Applicants acknowledge the drawing informalities noted by the Office and intend to remedy such informalities upon receiving a notice of allowable subject matter from the Office.

The Office objects to the drawings on grounds that there are no Y-axis figure legends appearing in Figs. 34A-34D, 39, 40, 50A-50B, 55A-55C or 58A-58B. Applicants herewith amend the drawings to include the figure legend "Neutrophil Migration Index" on the Y-axis appearing in each of Figs. 34A-34D, 39, 40, 50A-50B, 55A-55C and 58A-58B. The amended figure legends are consistent with those appearing in Fig. 6 and Fig. 7. The data shown in Figs. 6, 7, 34A-34D, 39, 40, 50A-50B, 55A-55C and 58A-58B were generated using the neutrophil chemotaxis inhibition assay procedure essentially as described in Example B.2 (on page 230, line 20 to page 231, line 16 of the specification). Thus, no new matter is believed to be added by the present amendment to the drawings.

Specification Informalities

The Office objects to the disclosure on grounds that an incorrect address is provided for the ATCC on page 229 of the specification. As amended herein, page 229 of the specification now specifies the correct address for the ATCC, as requested by the Office.

The Brief Description of the Drawings section of the specification does not correctly reflect the panels of Fig. 48. While Fig. 48 contains panels A-Z, the text in question refers to Figs. 48A-48T. Applicants herein amend the text in question to refer to Figs. 48A-48Z. No new matter is believed to be added hereby.

Rejections under 35 U.S.C. §112, second paragraph

Claims 1-30 are rejected under 35 U.S.C. §112, second paragraph as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention. The Office apparently finds that the claims are indefinite for reciting the term "apparent size" with respect to the claimed conjugate. The Office contends that it is not clear how the "apparent size" of the claimed conjugate is

determined.

Applicants respectfully traverse the rejection. The specification provides a precise definition of how to calculate the "apparent size" of the conjugate as that term is used in the specification and claims. In particular, the specification provides that "the terms 'hydrodynamic size', 'apparent size', 'apparent molecular weight', 'effective size' and 'effective molecular weight' of a molecule are used synonymously herein [to] refer to the size of a molecule as determined by comparison to a standard curve produced with globular protein molecular weight standards in a size exclusion chromatography system, wherein the standard curve is created by mapping the actual molecular weight of each standard against its elution time observed in the size exclusion chromatography system. Thus, the apparent size of a test molecule is derived by using the molecule's elution time to extrapolate a putative molecular weight from the standard curve." (Lines 19-26, page 39 of the specification.)

Since the above-quoted definition provides a particular method by which "apparent size" is to be calculated, the term "apparent size" is sufficiently clear and definite as used in the specification and claims. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §112, second paragraph.

Claim 14 is rejected as allegedly being indefinite for reciting "derived from a parental antibody". The Office apparently takes the position that the term "derived" has no universally accepted meaning and is not defined in the specification.

Claim 14 is now canceled. Thus, the rejection is moot with respect to this claim.

Claim 1 is amended to incorporate the elements of claim 14. Although Applicants do not necessarily agree with the rejection of claim 14, amended claim 1 recites "corresponding to a portion of a parental antibody" in place of the "derived from a parental antibody" language objected to by the Office. Since the "derived from" term objected to by the Office is no longer present in the claims, Applicants submit that the claim amendments overcome the rejection and respectfully request that it be withdrawn.

Rejection under 35 U.S.C. §112, first paragraph

Claim 14 is rejected under 35 U.S.C. §112, first paragraph as allegedly containing subject matter which was not described in the specification in such a way as to enable one

skilled in the art to make and use the invention. In particular, the Office takes the position that the specification did not enable the replacement of the cysteine residue with any amino acid as claimed. The Office apparently finds that one skilled in the art would not have expected that all such replacements to result in proper folding and packing of the heavy and light chains in the absence of the disulfide linkage between the chains. In addition, the Office notes that the specification taught no example in which the conjugate claimed in claim 14 was made and demonstrated to bind antigen. Finally, the Office cites European Patent Publication No. 0239400 to Winter (hereafter "Winter") for its apparent depiction of three disulfide bonds between the light and heavy chains of IgG. Based on the teaching of Winter, the Office finds it unclear which disulfide bond can be avoided without loss of antigen-binding activity. Accordingly, the Office concludes that the practitioner could have not made and used the claimed conjugates without undue experimentation.

Applicants respectfully traverse the rejection. The Office's position appears to rest on the Office's assumption that the native amino acid sequences and disulfide linkage of the light and heavy chain constant domains of an antibody fragment are essential for antigen-binding activity. Contrary to the Office's assumption, it is evident from the literature that the variable domains of the light and heavy chains will non-covalently associate to form an antigen-binding site without the support of native antibody sequence or structure outside of the variable domains. This principle is demonstrated by single chain Fv (scFv) phage display technologies. In a scFv phage display system, a heavy chain variable domain (fused to a phage coat protein) and a light chain variable domain are joined through a flexible linker to form a single polypeptide that is displayed on the surface of the phage clone. The antigen-binding ability of the scFv fragment displayed by the phage clone is used to select for and isolate the clone.

For example, McCafferty, et al., Nature, 348: 552-554 (1990), hereafter "McCafferty," newly cited, a copy of which is attached to the Information Disclosure Statement submitted herewith, demonstrated that the antigen binding activity and specificity of anti-lysozyme antibody D1.3 could be retained by an scFv fragment cloned into an fd phage vector. McCafferty reported that the phage display scFv exhibited the same pattern of reactivity and specificity as the D1.3 antibody (see Fig. 2 and the paragraph bridging the columns on page 553). In addition, McCafferty taught that the heavy and light chain variable domains of the

D1.3 antibody associate tightly as an Fv fragment and bind to antigen with a similar affinity to that of the parent antibody (see the first sentence of the main text on page 552).

Since the Fv and scFv fragments of the D1.3 antibody contain no constant domain sequence (and the scFv fragment actually contains phage coat protein sequence in place of the heavy chain constant domain), and since the Fv and scFv fragments exhibit the same antigen-binding activity as the parental D1.3 antibody, the data of McCafferty indicate that the presence of a native constant domain is unnecessary for the retention of antigen-binding activity in an antibody fragment. As shown below, the heavy and light chains of an intact antibody are covalently linked through a single disulfide bridge between the heavy chain constant domain and the light chain constant domain. Since the Fv and scFv fragments of McCafferty retain antigen-binding activity despite the complete absence of a disulfide linkage between the light and heavy chains, the record does not support the Office's assumption that the native amino acid sequences and disulfide linkage of the light and heavy chain constant domains of an antibody fragment are essential for antigen-binding activity.

Moreover, the teachings of McCafferty demonstrate that an example of the cysteine substitution recited in the claims is not required to enable the practitioner to make conjugates with antigen-binding activity as claimed. McCafferty taught that the light and heavy chain variable domains will tightly associate and exhibit antigen-binding activity even in the complete absence of constant domain sequence or when fused to a totally unrelated protein in place of constant domain sequence. Given that only a single amino acid residue is substituted with a non-native residue in the constant domain portion of the claimed conjugate, as compared to the total deletion or replacement of the constant domain sequence in the Fv and scFv constructs of McCafferty, the practitioner would reasonably expect that the claimed conjugates would exhibit at least as much antigen-binding activity as the Fv and scFv constructs of McCafferty in comparison to intact parental antibody.

In addition, the "diabody" system of Holliger, et al., Proc. Natl. Acad. Sci. (USA), 90: 6444-6448 (1993), hereafter "Holliger," newly cited, a copy of which is attached to the Information Disclosure Statement provided herewith, demonstrated that non-native sequence can be fused to the light and heavy chain variable domains that form an antigen binding site without loss of antigen-binding activity (see the second full paragraph and Table 1 on page

6446). Holliger taught that a bispecific "diabody" can be made by linking the variable heavy domain (V_H) and variable light domain (V_L) of two different antibodies together on a single polypeptide chain. By using a linker that is too short to allow pairing between two domains on the same chain, the domains are forced to pair with complementary domains on another chain, thereby creating a heterodimer with two different antigen-binding sites. In the bispecific diabody system of Holliger, the light chain variable domain of antibody A is fused directly to the heavy chain variable domain of antibody B and the light chain variable domain of antibody B is fused directly to the heavy chain variable domain of antibody A.

Despite its replacement of the native constant domain sequence of antibody A with the variable domain sequence of antibody B and its replacement of the native constant domain sequence of antibody B with the variable domain sequence of antibody A, the bispecific antibody of Holliger retains the antigen-binding activities of both antibodies A and B. Whereas the claimed conjugate substitutes a single amino acid residue in the constant domain portion of the antibody fragment in the conjugate, Holliger's bispecific diabodies substitute all of the constant domain sequence with variable domain sequence from an unrelated antibody. Since Holliger's bispecific diabodies employ a far greater and more drastic change to the constant domain and the antibody structure in general than that found in the antibody fragments of the claimed conjugates, and since the bispecific diabodies exhibit no loss of antigen-binding activity, the practitioner would reasonably expect the antibody fragments in the claimed conjugates to retain useful antigen-binding activity.

Finally, Applicants respectfully submit that the positions of two of the three disulfide bonds depicted in Fig. 1 of Winter are inaccurate. Winter described Fig. 1 as a depiction of IgG (see line 5, page 6 of Winter). However, U.S. Pat. No. 5,618,920 to Robinson, hereafter "Robinson," newly cited, a copy of which is attached to the Information Disclosure Statement submitted herewith, provided a far more detailed portrayal of IgG1 in which a single disulfide bond covalently links the light and heavy chains and two intra-chain disulfide bonds appear in each chain (see Fig. 31 and the text in col. 10, lines 13-14). From the greater detail of the IgG1 molecule depicted in Fig. 31 of Robinson, it appears that Fig. 1 of Winter mistakenly represented the intrachain disulfides as interchain disulfides.

In view of the teachings of Robinson, there is only a single disulfide bond that is

avoided in the antibody fragment of the claimed conjugate. Since the practitioner would have reasonably expected that the recited antibody fragment will retain antigen-binding activity, as shown above, the specification would have enabled the practitioner to make and use the claimed conjugate.

In view of the above, Applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph.

Double Patenting Rejection based on U.S.S.N. 09/234,182

The Office provisionally rejects claims 1-37 under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 1, 5, 8, 10, 15, 18, 19, 21, 24, 25 and 30-35 of copending application U.S. Ser. No. 09/234,182. Although Applicants do not necessarily agree with the rejection, Applicants submit herewith a Terminal Disclaimer that terminally disclaims the period of any patent granted on the present application that would extend beyond the term of any patent granted on application U.S. Ser. No. 09/234,182. Since the Terminal Disclaimer overcomes the rejection, Applicants respectfully request that it be withdrawn.

Double Patenting Rejection based on U.S.S.N. 09/355,014

The Office provisionally rejects claims 1-37 under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 1-25 and 28-31 of copending application U.S. Ser. No. 09/355,014. Although Applicants do not necessarily agree with the rejection, Applicants submit herewith a Terminal Disclaimer that terminally disclaims the period of any patent granted on the present application that would extend beyond the term of any patent granted on application U.S. Ser. No. 09/355,014. Since the Terminal Disclaimer overcomes the rejection, Applicants respectfully request that it be withdrawn.

Double Patenting Rejection based on U.S.S.N. 09/012,116

The Office provisionally rejects claims 1-37 under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 1-30 of

copending application U.S. Ser. No. 09/012,116. Application U.S. Ser. No. 09/012,116 is now abandoned. Since the provisional rejection is moot, Applicants respectfully request that it be withdrawn.

Rejection under 35 U.S.C. §103(a) based on Faanes

Claims 1-13, 15-16, 18-24, 26-33, and 36-37 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over U.S. Pat. No. 5,695,760 to Faanes, et al., hereafter "Faanes."

Although Applicants do not necessarily agree with the rejection, Applicants have amended claim 1 (and necessarily also remaining claims 2-7, 9-11, 13, 15, 16, 18-24, 26-33, and 36-37 depending therefrom) to recite a conjugate having at least one antibody fragment that is derivatized with a nonproteinaceous polymer through the covalent attachment of the polymer to a cysteine residue in one chain that would be in a disulfide bridge with a corresponding cysteine residue in the opposite chain but for substitution of the corresponding cysteine residue with another amino acid residue. Thus, the amended claims are directed to polymer derivatization at a unique site in the antibody fragment that is created by the replacement of a native cysteine residue with another amino acid residue in the molecule.

To anticipate a claim in a patent application, a prior art reference must teach every element of the claim (MPEP 2131). Faanes does not teach or suggest the creation of a unique polymer-derivatization site through the replacement of a native cysteine residue with another amino acid residue in any of the enlimomab (anti-ICAM monoclonal antibody)-PEG conjugates of Faanes. Since Faanes did not provide the unique polymer-derivatization site specified in the claims, Faanes does not anticipate the claimed invention.

In view of the present claim amendments and the foregoing arguments, Applicants respectfully request reconsideration and withdrawal of the §103(a) rejection based on Faanes.

Rejection under 35 U.S.C. §103(a) based on Faanes and Zapata

Claims 1-13, 15-33, and 36-37 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Faanes in view of Zapata, et al., FASB J., 9: A1476 (1995), hereafter "Zapata."

Without intending acquiescence to the rejection, but rather to expedite prosecution,

Applicants have amended claim 1 (and necessarily also remaining claims 2-7, 9-11, 13, 15, 16, 18-24, 26-33, and 36-37 depending therefrom) to recite a conjugate having at least one antibody fragment that is derivatized with a nonproteinaceous polymer through the covalent attachment of the polymer to a cysteine residue in one chain that would be in a disulfide bridge with a corresponding cysteine residue in the opposite chain but for substitution of the corresponding cysteine residue with another amino acid residue. Thus, the amended claims are directed to polymer derivatization at a unique site in the antibody fragment that is created by the replacement of a native cysteine residue with another amino acid residue in the molecule.

Faanes does not teach or suggest the creation of a unique polymer-derivatization site through the replacement of a native cysteine residue with another amino acid residue in any of the enlimomab (anti-ICAM monoclonal antibody)-PEG conjugates of Faanes. Zapata taught the PEGylation of the free thiol in the hinge region of an anti-CD18 Fab'. Zapata provided no teaching or suggestion to alter the disulfide bridge between the light and heavy chains of the Fab' molecule. Since nothing in the cited references would have provided the practitioner with the unique polymer-derivatization site recited in the claims, the claimed invention is patentable over any combination of Faanes and Zapata.

In view of the claim amendments and the foregoing arguments, Applicants respectfully request reconsideration and withdrawal of the §103(a) rejection based on Faanes and Zapata.

Rejection under 35 U.S.C. §103(a) based on Faanes and Harlow

Claims 1 and 34-35 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable Faanes and further in view of Harlow,et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratories, pp. 324-339, 1988), hereafter "Harlow."

Without intending acquiescence to the rejection, but rather to expedite prosecution, Applicants have amended claim 1 (and necessarily also remaining claims 34-35 depending therefrom) to recite a conjugate having at least one antibody fragment that is derivatized with a nonproteinaceous polymer through the covalent attachment of the polymer to a cysteine residue in one chain that would be in a disulfide bridge with a corresponding cysteine residue in the opposite chain but for substitution of the corresponding cysteine residue with another

amino acid residue. Thus, the amended claims are directed to polymer derivatization at a unique site in the antibody fragment that is created by the replacement of a native cysteine residue with another amino acid residue in the molecule.

Faanes fails to teach or suggest the unique polymer-derivatization site recited in the claims, as shown above. In addition, Harlow contains no description that would have led the practitioner to employ the unique polymer-derivatization site in an antibody fragment. Since nothing in the cited references would have provided the practitioner with the unique polymer-derivatization site recited in the claims, the claimed invention is patentable over any combination of Faanes and Harlow.

In view of the claim amendments and the foregoing arguments, Applicants respectfully request reconsideration and withdrawal of the §103(a) rejection based on Faanes and Harlow.

In light of the above, Applicants respectfully submit that the application is in condition for allowance and earnestly solicit a Notice to that effect. If the Examiner has any question concerning this response, the Examiner should not hesitate to contact the undersigned attorney at the telephone number indicated below.

Respectfully submitted,
GENENTECH, INC.

Date: November 22, 2000 By: 
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Fig. 42 depicts the nucleic acid sequences of the stop template primer (SEQ ID NO: 63) and the NNS randomization primer (SEQ ID NO: 64) used for random mutagenesis of amino acid position 35 in variable light chain CDR-L1 of humanized antibody 6G4V11.

Fig. 43A is a table of data describing the frequencies of different phage display clones obtained from the randomization of amino acid position 35 in variable light chain CDR-L1 of humanized antibody 6G4V11.

Figs. 43B, 43C, 43D and 43E are graphs of displacement curves depicting the inhibition of ^{125}I-IL-8 binding to neutrophils exhibited by the 6G4V11N35A, 6G4V11N35D, 6G4V11N35E and 6G4V11N35G Fab's.

Fig. 44 contains a graph depicting the typical kinetics of an anti-IL-8 antibody fragment (6G4V11N35A F(ab')$_2$) binding to IL-8. Fig. 44 also contains a table of data providing the equilibrium constant for 6G4V11N35A Fab binding to IL-8 (rate constants were not determined "ND"), and the equilibrium and rate constants for 6G4V11N35A F(ab')$_2$ and 6G4V11N35E Fab binding to IL-8.

Fig. 45 depicts the DNA sequence (SEQ ID NO: 65) and amino acid sequence (SEQ ID NO: 62) of the 6G4V11N35E light chain in an N-terminal fusion with the STII leader peptide. Complementarity determining regions L1, L2 and L3 are underlined.

Fig. 46 is a graph depicting the ability of 6G4V11N35E Fab to inhibit human IL-8 (dark columns) and rabbit IL-8 (light columns) mediated neutrophil chemotaxis. Data are presented for 6G4V11N35E Fab samples at concentrations of 0.4, 1.2, 3.7, 11 and 33 nM, and for an isotype control antibody (4D5) sample at a concentration of 100 nM, in the presence of 2 nM human IL-8 or 2 nM rabbit IL-8. In addition, inhibition data are presented for a no IL-8 buffer control sample (denoted "Buffer") and for human and rabbit IL-8 control samples (denoted "IL-8").

Fig. 47 depicts the DNA sequence of the sense (SEQ ID NO: 66) and anti-sense (SEQ ID NO: 67) strands of a PvuII-XhoI synthetic nucleotide encoding amino acids Leu4 to Phe29 of the 6G4V11N35A heavy chain.

Figs. 48A-48Zdepict the DNA sequence (SEQ ID NO: 68) of plasmid

(Substitute Sheet)

hours at 25°C. The tubes were centrifuged (210 X g, 2-8°C) for 10 min, and supernatants were transferred to 96 well tissue culture plates (30 µl/well). Elastase substrate stock, 10 mM methoxysuccinyl-alanyl-alanyl-propyl-valyl-p-nitroanilide (Calbiochem, La Jolla, CA) in DMSO was prepared and stored at 2-8°C. Elastase substrate solution (1.2 mM substrate, 1.2 M NaCl (Mallinckrodt, Paris, Kentucky), 0.12 M HEPES pH 7.2 in distilled water) was added (170 µl/well) to the supernatants and incubated for 0.5 to 2 hours at 37°C (until control O.D. of 1.0 was reached). Absorbance was measured at 405 nm (SLT 340 ATTC plate reader, SLT Lab Instruments, Austria).

The results are shown in Figure 1. At a 1:1 ratio of IL-8 to mAb 5.12.14, the antibody was able to effectively block the release of elastase from neutrophils.

The hybridoma producing antibody 5.12.14 was deposited on February 15, 1993 with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, U.S.A. (ATCC) and assigned ATTC Accession No. HB 11553.

B2

B. GENERATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST RABBIT IL-8

Antibodies against rabbit IL-8 were generated in essentially the same process as anti-human IL-8 antibodies using rabbit IL-8 as immunogen (kindly provided by C. Broaddus; see also Yoshimura *et al.* J. Immunol. 146:3483 (1991)). The antibody was characterized as described above for binding to other cytokines coated onto ELISA plates; no measurable binding was found to MGSA, fMLP, C5a, b-TG, TNF, PF4, or IL-1.

The hybridoma producing antibody 6G4.2.5 was deposited on September 28, 1994, with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, USA. (ATCC) and assigned ATTC Accession No. HB 11722.

Recombinant human-murine chimeric Fabs for 5.12.14 and 6G4.2.5 were constructed as described below. A chimeric 6G.4.25 Fab is compared with a chimeric 5.12.14 Fab in detail below.

(Substitute Sheet)

Fig. 42 depicts the nucleic acid sequences of the stop template primer (SEQ ID NO: 63) and the NNS randomization primer (SEQ ID NO: 64) used for random mutagenesis of amino acid position 35 in variable light chain CDR-L1 of humanized antibody 6G4V11.

Fig. 43A is a table of data describing the frequencies of different phage display clones obtained from the randomization of amino acid position 35 in variable light chain CDR-L1 of humanized antibody 6G4V11.

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Fig. 47 depicts the DNA sequence of the sense (SEQ ID NO: 66) and anti-sense (SEQ ID NO: 67) strands of a PvuII-XhoI synthetic nucleotide encoding amino acids Leu4 to Phe29 of the 6G4V11N35A heavy chain.

Figs. 48A ~~{48T depict}~~ **[48Zdepict]** the DNA sequence (SEQ ID NO: 68) of plasmid

hours at 25°C. The tubes were centrifuged (210 X g, 2-8°C) for 10 min, and supernatants were transferred to 96 well tissue culture plates (30 µl/well). Elastase substrate stock, 10 mM methoxysuccinyl-alanyl-alanyl-propyl-valyl-p-nitroanilide (Calbiochem, La Jolla, CA) in DMSO was prepared and stored at 2-8°C. Elastase substrate solution (1.2 mM substrate, 1.2 M NaCl (Mallinckrodt, Paris, Kentucky), 0.12 M HEPES pH 7.2 in distilled water) was added (170 µl/well) to the supernatants and incubated for 0.5 to 2 hours at 37°C (until control O.D. of 1.0 was reached). Absorbance was measured at 405 nm (SLT 340 ATTC plate reader, SLT Lab Instruments, Austria).

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The hybridoma producing antibody 5.12.14 was deposited on February 15, 1993 with the American Type Culture Collection, ~~{12301 Parklawn Drive, Rockville, MD}~~ **[10801 University Boulevard, Manassas, VA 20110-2209]**, U.S.A. (ATCC) and assigned ATTC Accession No. HB 11553.

B. GENERATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST RABBIT IL-8

Antibodies against rabbit IL-8 were generated in essentially the same process as anti-human IL-8 antibodies using rabbit IL-8 as immunogen (kindly provided by C. Broaddus; see also Yoshimura *et al.* J. Immunol. 146:3483 (1991)). The antibody was characterized as described above for binding to other cytokines coated onto ELISA plates; no measurable binding was found to MGSA, fMLP, C5a, b-TG, TNF, PF4, or IL-1.

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Recombinant human-murine chimeric Fabs for 5.12.14 and 6G4.2.5 were constructed as described below. A chimeric 6G.4.25 Fab is compared with a chimeric 5.12.14 Fab in detail below.

Marked-Up Copy of Amended Claims of U.S. Ser. No. 09/489,394 (2/22/01 Amendment)

1. (Amended) A conjugate consisting essentially of at least one [or more] antibody fragment[s] covalently attached to [one or] no more than about 2 nonproteinaceous polymer molecules, wherein the apparent size of the conjugate is at least about 500 kD, wherein the antibody fragment comprises a heavy chain and a light chain corresponding to a portion of a parental antibody, wherein in the portion of the parental antibody the heavy and light chains are covalently linked by a disulfide bond between a cysteine residue in the light chain and a cysteine residue in the heavy chain, wherein in the antibody fragment the cysteine residue in the light or heavy chain is substituted with another amino acid and the cysteine residue in the opposite chain is covalently linked to a nonproteinaceous polymer molecule, and wherein at least one antibody fragment comprises an antigen binding site that binds to a polypeptide selected from the group consisting of: human vascular endothelial growth factor (VEGF), human p185 receptor-like tyrosine kinase (HER2), human CD20, human CD18, human CD11a, human IgE, human Apo-2 receptor, human tumor necrosis factor-α (TNF-α), human tissue factor (TF), human α4β7 integrin, human GPIIb-IIIa integrin, human epidermal growth factor receptor (EGFR), human CD3, and human interleukin-2 receptor α-chain (TAC).

9. (Amended) The conjugate of claim [8] 1 wherein the antibody fragment is $F(ab')_2$.

15. (Amended) The conjugate of claim [8] 1 wherein the antibody fragment is selected from the group consisting of Fab, Fab' and Fab'-SH.